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# Storage Proteins Accumulation and Aggregation in Developing Wheat Grains

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## Abstract

The aggregative properties of wheat grain prolamins are largely responsible for the technological functionalities of the flours and doughs. The ability of wheat prolamins to form a plastic three-dimensional network during the mixing depends to a large extent on their ability to interact. These aggregative properties, which can be evaluated by measuring their molecular weight distribution, are dependent on the polymorphism of the protein subunits present but also on the environmental conditions that are applied during grain development. Much progress has been made in the last 30 years at a genetic level to better understand and/or to favour the interaction properties of the storage proteins. However, these improvements can be strongly limited by environmental conditions. Any modification of the redox status of the grain cells in response to an oxidative stress can lead to a decrease in the degree of association of the prolamins by limiting the protein-protein interactions during the grain desiccation. Considering the current and projected environmental impacts (i.e. climate change with increasing heat stress), it is essential to better understand these phenomena to implement new breeding strategies for a sustainable quality.

**Keywords:** wheat, storage proteins, aggregation, breadmaking quality

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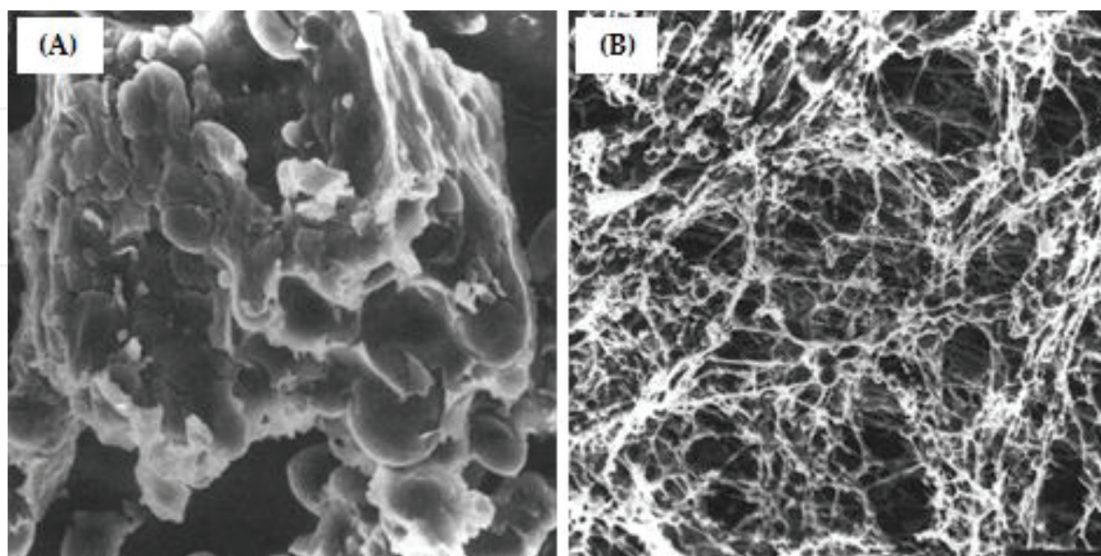
## 1. Introduction

During the last 60 years, in the field of cereal chemistry, the scientific community has been working to determine in an ever more precise way, the nature of the constituents responsible for the acquisition of technological properties (i.e. breadmaking properties for common wheat doughs and/or pasta properties in the case of durum wheat). Particular emphasis has been placed on those whose (quantitative and/or qualitative) variations account for observed and measured changes in processing ability.

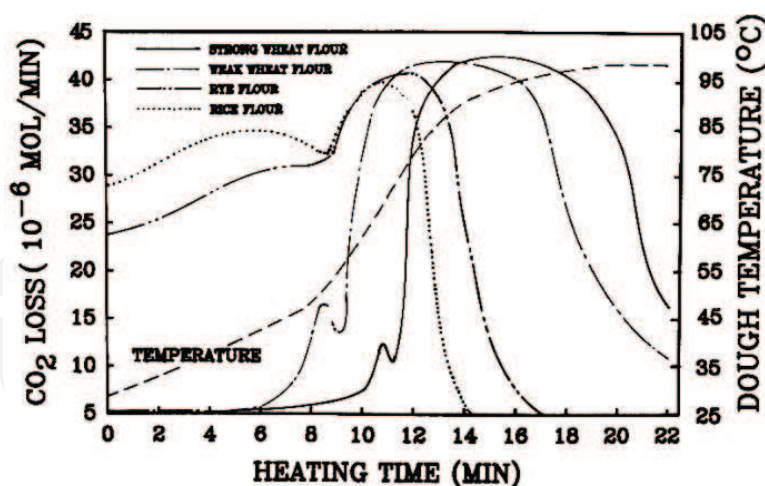
As early as the 1950s, thanks to very good recombination experiments with flour constituents, Finney [1] confirms that the baking capacity is essentially conferred by gluten. Gluten, which can be defined as a viscoelastic protein complex formed after hydration and the addition of flour, consists of a heterogeneous mixture of prolamins (i.e. gliadins and glutenins) associated with covalent (S-S) and non-covalent bonds (hydrogen, hydrophobic and ionic). The specific role of certain protein fractions (monomer to polymeric proteins ratio) in the different properties of wheat doughs was also highlighted.

During the period 1970–1990, it became clear that the variation of the baking capacity of a flour is based on the ability of its storage proteins (i.e. prolamins) to form, during mixing, a three-dimensional plastic structure (**Figure 1A and B**). This remarkable structure creates a cohesive and viscoelastic network, insoluble in water, ensuring the retention of carbon dioxide, ethanol and aromas, during the fermentation of the dough and unlike other cereals for which these properties are non-existent (**Figure 2**). Thus, a common wheat is all the more breadmaking that its storage proteins have a strong tendency to aggregate into a three-dimensional viscoelastic network during mixing. Thus, gluten is considered a transient network whose mechanical properties depend on the density of the junction zones between the elements that compose it [2].

Since the 1990s, thanks to the integration of many complementary scientific approaches (i.e. molecular biology, biochemistry, analytical chemistry, rheology, etc.), a clearer vision of the transformation processes and the role of the main protein constituents within them have begun to take shape [3]. Thus, attention has been focused on the (polymeric) glutenin fraction because a strong relationship has been established between breadmaking properties, such as mixing time, extensibility and loaf volume and the molecular weight distribution (MWD) of the polymeric protein components [4].



**Figure 1.** Scanning electron micrographs of durum wheat (A) flour and (B) dough particles (From Hoseney and Rogers [3]).



**Figure 2.** Loss of carbon dioxide and increase in temperature in relation to the heating time for different doughs of different cereal grains (From Hosney and Rogers [3]).

To the extent that any changes (genetically and/or environmentally controlled) in the molecular size and/or aggregation status of these polymeric proteins can potentially result in very significant changes in the technological properties of the products concerned, it is important to understand how they are synthesized and accumulated in grains of wheat during their development. This knowledge is essential if we are to manipulate wheat quality in the future for traditional or new end users.

This chapter reviews the definition of the molecular weight distribution of wheat storage proteins, their changes during grain development and the impacts of environmental factors.

## 2. Molecular weight distribution (MWD) of wheat storage proteins

### 2.1. Classification and polymorphism of wheat grain proteins

Like all grain seeds, wheat grain contains a large number of proteins classified as structural proteins, functional proteins and reserve proteins. They are unequally distributed within the different cell of the grain. A natural gradient of distribution can be highlighted. As a result, the starch to protein ratio significantly increases from the peripheral to the central regions of the grain. Given the relative weight of these different cells, 70–80% (w/w) of the proteins are in the albumen.

The classification system for cereal proteins is mainly based on Osborne's historical work, in 1907 [5], based on their differences in solubility later used in sequential extractions (**Table 1**). As a result, four major protein fractions have been defined: albumins (soluble in water), globulins (soluble in dilute salt solutions), gliadins (soluble in diluted alcohols, 70% ethanol) and finally, glutenins (residual proteins, partially soluble in diluted acids and bases). Other authors have enriched these classifications based on structural and/or functional properties [6, 7]. Within the large family of the storage proteins (prolamins), two main classes can be

Osborne [5] classification			Shewry et al. [6] classification		Singh and Shepherd [7] classification	
Protein fraction	Solubility	MW (kDa)	Composition	Structure	Gene localization*	Function
Albumins and globulins	Water neutral salts	5–90				Structural and functional proteins
Gliadins	Diluted alcohols	25–75		Monomers		Storage proteins (prolamins)
- $\omega$			Poor in S		<i>Gli-1</i> (1A,1B,1D)S	
- $\alpha$			Reach in S		<i>Gli-2</i> (6A,6B,6D)S	
- $\beta$					<i>Gli-2</i> (6A,6B,6D)S	
- $\gamma$					<i>Gli-1</i> (1A,1B,1D)S	
Glutenins	Acids, bases, reductants, detergents	100 to several millions	Reach in S	Polymers		
- LMW					<i>Glu-3</i> (1A,1B,1D)S	
- HMW					<i>Glu-1</i> (1A,1B,1D)L	

\**Allelic blocks*, wheat homologous chromosomes (noted 1–6), wheat genomes (A, B and D) and chromosome position: (S) short arm, (L) long arm.

**Table 1.** Classification of wheat grain proteins.

differentiated due, to their degree of aggregation/polymerization. Thus, on the one hand, gliadins (soluble monomeric proteins in aqueous alcohols), which represent approximately 30–40% (w/w) of flour proteins and on the other hand, glutenins representing, 40–45% (w/w) of the total flour proteins. The latter are polymeric and aggregated proteins, forming a much more complex material than the gliadins.

Gliadins correspond to a mixture of monomeric proteins of molecular weight between 25 and 75 kDa and are characterized by their richness in glutamine and proline. They represent 45% (w/w) of the total prolamins. There are four classes based on their electrophoretic behaviour (i.e. increasing mobility in acid medium):  $\alpha/\beta$ ,  $\gamma$  and  $\omega$ -gliadins (which, respectively, represent 44–60%, 30–46% and 6–20% of total gliadins) [8].

Glutenins, for their part, represent 40–50% (w/w) of total proteins; they are rich in proline and glutamic acid and their content in basic amino acids is higher than that of gliadins. They constitute a much more complex material formed of an assembly of polypeptide chains, commonly called subunits, linked together mainly by intermolecular disulphide bridges. These subunits have been grouped into two different subgroups: low molecular weight subunits (LMW-GS) and high molecular weight subunits (HMW-GS).

LMW-GS account for an average of two-thirds of total glutenins. They are very polymorphic and have molar masses between 30 and 50 kDa. Given their similarity to some gliadins, these have sometimes been difficult to quantify. HMW-GS, as their name indicates, have higher molecular weights ranging from 95 to 130 kDa. According to their SDS-PAGE migration, they fall into two groups: HMW-GS $\gamma$  (67–74 kDa) and HMW-GS $\chi$  (83–88 kDa).



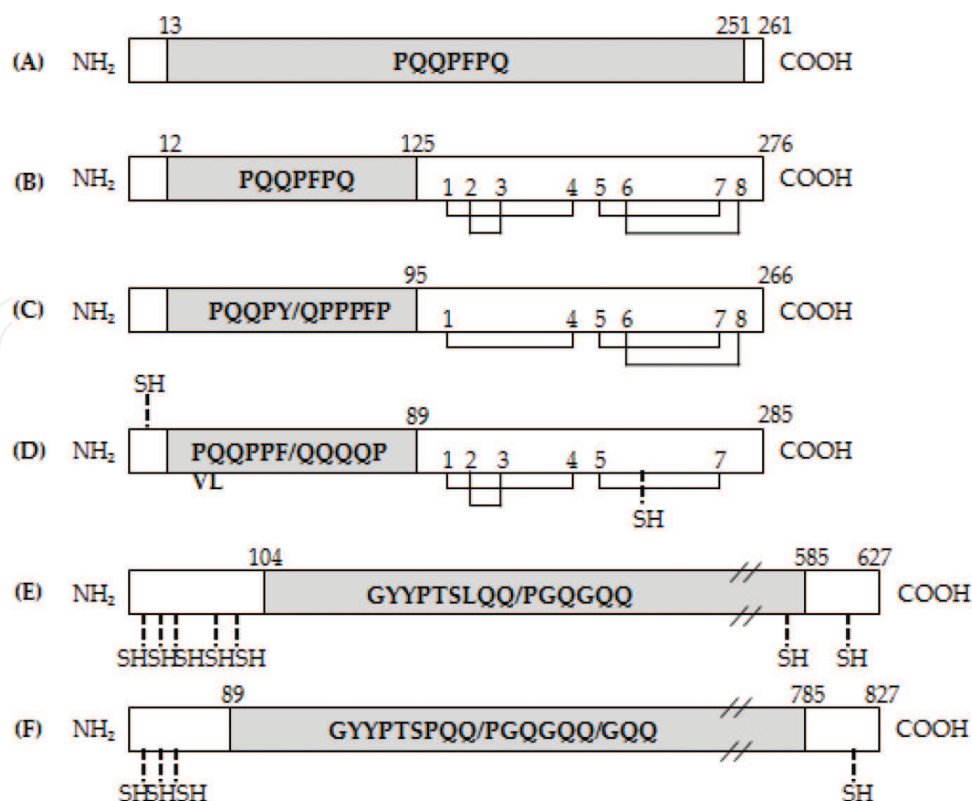
Gliadins have a large genetic polymorphism, it has been possible to detect between 20 and 40 different constituents for a wheat variety [9]. Within a class of gliadins, it is possible to find several sub-families depending on the composition and richness of certain amino acids (the  $\omega$ 1 and  $\omega$ 5 gliadins differ in basic amino acids, glutamine and proline, than that  $\gamma$ 1,  $\gamma$ 2 and  $\gamma$ 3 differ in their richness in tyrosine, lysine and methionine). Thus, the polymorphism of gliadins is very important that it serves as a basis for the varietal identification of wheat [10].

The polymorphism of low molecular weight glutenic subunits (LMW-GS) is less important than that of gliadins. For a given variety, there are 7–6 LMW-GS. But 40 different LMW-GS were found in 222 varieties of soft wheat [11]. Finally, high molecular weight glutenic subunits (HMW-GS) are the prolamins that have the lowest polymorphism. The association of two genes at each Glu-A1, Glu-B1 and Glu-D1 locus was noted. The  $\alpha$ -type genes express subunits of masses greater than those encoded by  $\gamma$ -type genes in SDS-PAGE [12]. However, some  $\gamma$ -type HMW-GS (notably subunit 12) have been shown to have important immunochemical similarities with  $\alpha/\beta$  and  $\gamma$ -gliadins [13]. In all cases, recombination between these genes is very rare. The different wheat varieties contain between 3 and 5 HMW-GS. Indeed, 1Ay genes are never expressed, and 1By and 1Ax genes are only expressed in some varieties [14].

The primary structure of the storage proteins is well understood. They comprise three distinct domains (**Figure 3**): a central domain made up of repeated sequences and two domains formed of non-repeated sequences at the ends (i.e. C- and N-terminal). The understanding of these sequences has made it possible to locate particularly important cysteine residues because of their ability to form disulphide bonds (intra and/or intermolecular).  $\alpha$ -,  $\beta$ - and  $\gamma$ -gliadins are provided with cysteines at their C-terminal domains; these all being involved in the formation of intramolecular disulphide bridges. HMW-GS have unpaired cysteine in their C-terminal domain and several others in their N-terminal domain; LMW-GS carrying seven C-terminal cysteines and one N-terminal cysteine. Thanks to these unpaired cysteines, unlike gliadins, HMW-GS and LMW-GS are able to form intermolecular disulphide bridges. Some authors report a globular type structure for the N- and C-terminal and a spiral structure for the repetitive domain (**Figure 3**).

## 2.2. Gliadin to glutenin ratio

Generally, it is accepted that the functional properties of gluten proteins are related to their ability to form a network during technological processes [17, 18]. However, gliadins and glutenins do not have the same effect on the rheological properties of doughs or glutes. Consequently, gliadins explain the viscous nature, while glutenins determine elasticity. In fact, the small quantity of cysteine residues in these storage proteins makes it possible to establish an important structural and functional distribution between gliadins and glutenins (**Figure 4**). For the former, all cysteine residues are involved in the establishment of intramolecular disulphide bridges while for both high and low molecular weight glutenins, a number of cysteines not involved in intramolecular bonds are therefore available to establish intermolecular links with other subunits. Glutenins are therefore likely to constitute polymers with a real consistency, thanks to the formation of intermolecular disulphide bridges, while gliadins remain in the monomeric state. The latter may, however, be aggregated by weak bonds

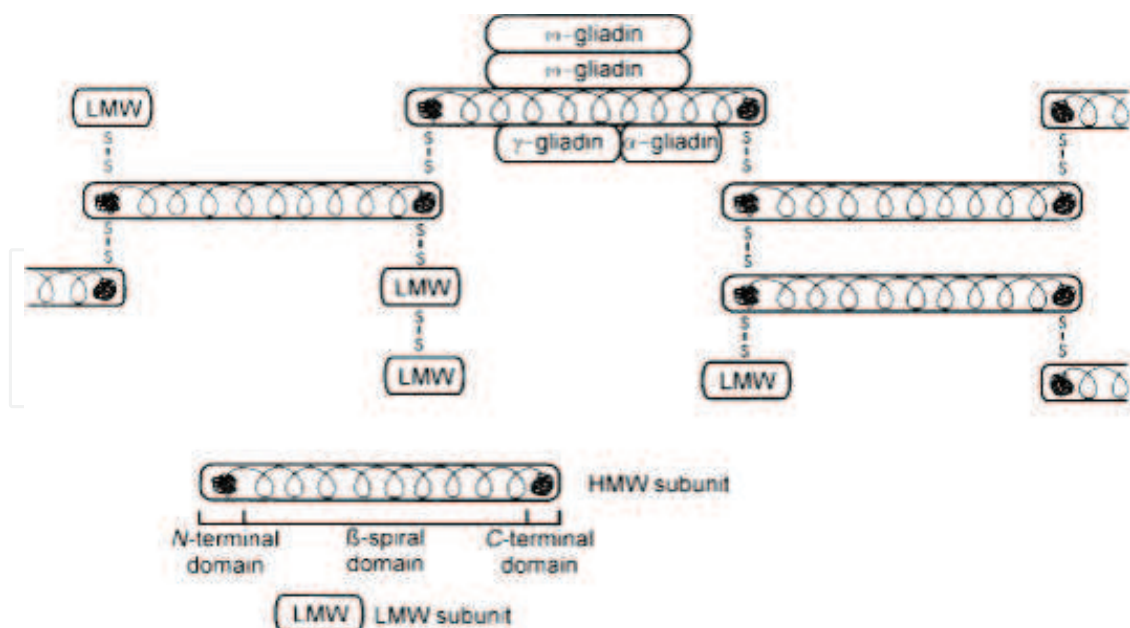


**Figure 3.** Schematic structures of typical primary structures of (A)  $\omega$ -gliadin, (B)  $\alpha$ -gliadin, (C)  $\gamma$ -gliadin, (D) LMW-GS, (E) HMW-GS $\gamma$  and (F) HMW-GS $\alpha$  [15, 16]. Repetitive sequences are shaded and disulphide bonds between conserved cysteine residues (1–8) in the  $\gamma$ -gliadin are shown as lines. SH denotes the positions of cysteine residues in the HMW prolamins. Single letter abbreviations for amino acids: F = phenylalanine; G = glycine; L = leucine; P = proline; Q = glutamine; S = serine; V = valine and Y = tyrosine.

(hydrogen and hydrophobic). The viscoelasticity of gluten depends on its state of polymerization and the interactions between polymers [2].

A large number of conventional fractionation and reconstitution tests have been conducted based on the differential physical properties observed in purified gliadins and glutenins. The aim of these studies was to link variations in molecular weight distribution (i.e. monomer to polymer ratio) with the rheological characteristics of the glutens obtained. In the majority of cases, the results obtained during these different reconstitution studies have demonstrated that the rheological properties of the restructured flours generated are strongly influenced by the ratio of these two protein fractions [20, 21]. With a constant amount of prolamins, the strength of the reconstituted flour, measured at the time of the dough making with a mixograph (i.e. peak time value mix (MPT)), is related to the proportion of polymeric proteins.

The development of the original analytical approaches (i.e. high performance liquid chromatography of size exclusion, SEC-HPLC) during the 1980s confirmed the vast majority of these hypothesis, which were essentially based on results obtained from differential solubility protocols (i.e. gliadins vs. glutenins). Thus, many authors [22–29] have confirmed the existence of a significant relationship between the relative amount of glutenin aggregates and the baking quality of many everyday wheat genotypes.



**Figure 4.** A structural model for wheat gluten in which the HMW subunits provide a disulphide-bonded backbone which interacts with other gluten proteins through disulphide bonds (LMW subunits) and non-covalent interactions (gliadins) (From Shewry et al. [19]).

The molecular weight distribution (MWD) of prolamins is becoming recognized as the main determinant of physical dough properties [30, 31]. However, in theory, the MWD can be altered from one sample of wheat (or one cultivar) to another by changes in the relative proportions of monomeric proteins and polymeric proteins (gliadins to glutenins ratio) but also by changes in the size distribution of polymeric proteins [32].

### 2.3. Size distribution of polymeric proteins

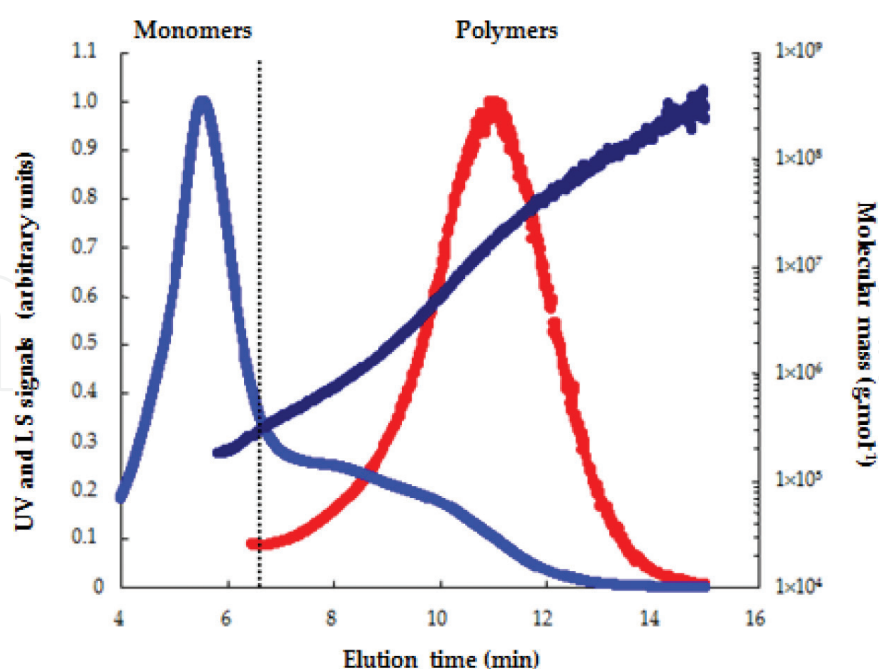
Chen and Bushuk [33] revealed that part of the glutenin is soluble in acetic acid thus making the distinction between an insoluble and a soluble fraction. The importance of this distinction became clear when Orth and Bushuk [34] demonstrated a positive correlation between the amount of acetic acid insoluble glutenin and bread loaf volume. From then on, insoluble glutenin became widely recognized as the key protein fraction that can explain differences in dough strength and breadmaking quality [35]. The use of detergents (SDS) and organic solvents (propanol) [36] allowed an even better separation and led to the conclusion that insolubility was due to size and a very high degree of polymerization. Other groups developed methodology with propanol to further separate soluble protein parts from the insoluble glutenin. Currently, two main methods are in use to quantify and characterize this fraction. The first corresponds to the so-called unextractable polymeric protein (UPP) method using propanol and during which unextractable polymeric protein (UPP) fraction is obtained. Upon sonication, this fraction becomes soluble in SDS [28, 29] and can be analysed using size exclusion chromatography [27, 37]. The other method is the SDS method as advanced by Graveland et al. [38] resulting in the SDS-insoluble gel protein fraction. This fraction was renamed glutenin macro polymer (GMP) to reflect its highly aggregated nature [39, 40]. Moonen et al. [41]



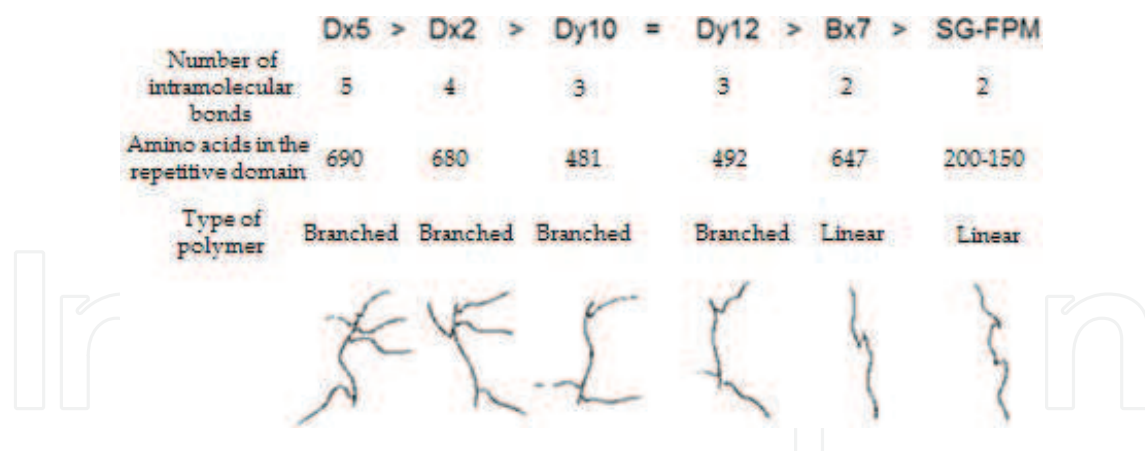
found that the SDS-insoluble glutenin-gel protein fraction highly correlated with SDS sedimentation values and loaf volume. Weegels et al. [40, 42] studied this fraction in great detail and presented firm evidence that GMP quantity correlates to bread loaf volume.

In addition to these classical approaches (UPP and/or GMP), new analytical protocols have been developed since the early 2000s to separate and more accurately characterize the molecular size distribution of the polymeric proteins. Flow field-flow fractionation (FFFF) [43–46], which is a new separation technique without any stationary phase, and which is therefore not hampered by a steric exclusion limit [47–49], has been used successfully to separate a number of HMW fractions [50–52]. Furthermore, the MALLS technique which is one of the most effective means of determining molecular weight, size and conformation of glutenin polymers without reference to standards [48, 53–56] has been applied in combination with the A-FFFF method to accurately measure size and conformation of wheat glutenins [57] (Figure 5).

The glutenin association level (i.e. the size distribution) is strongly correlated with the HMW-GS/LMW-GS [58–60] ratio and the nature of the HMW-GS present (especially HMW-GS pair 5 + 10 vs. HMW-GS pair 2 + 12 coded by *Glu-D1*). As demonstrated by the different experimental approaches carried out in recent years [61–64], the different glutenin subunits (i.e. HMW-GS, LMW-GS and HMW-GS  $\alpha$  and  $\gamma$  type) are unequally distributed within polymers. These results demonstrate the existence of a highly ordered structure in which some subunits play a predominant role, notably because of their difference in functionality (i.e. number and especially position of cysteine residues capable of forming intermolecular bonds) [65] (Figure 6).



**Figure 5.** Asymmetrical flow field-flow fractionation (A4F) profiles of total solubilized storage proteins of a common French wheat cultivar (Soissons). UV (blue line), light scattering at 90° (red line) and molecular weight in relation to elution time (dark line) (from Lemelin et al. [57]).



**Figure 6.** Hierarchical arrangement of HMW-GS and LMW-GS in relation to their intrinsic contribution to polymer formation (from Kasarda [65]).

### 3. Accumulation of prolamins in developing wheat grains

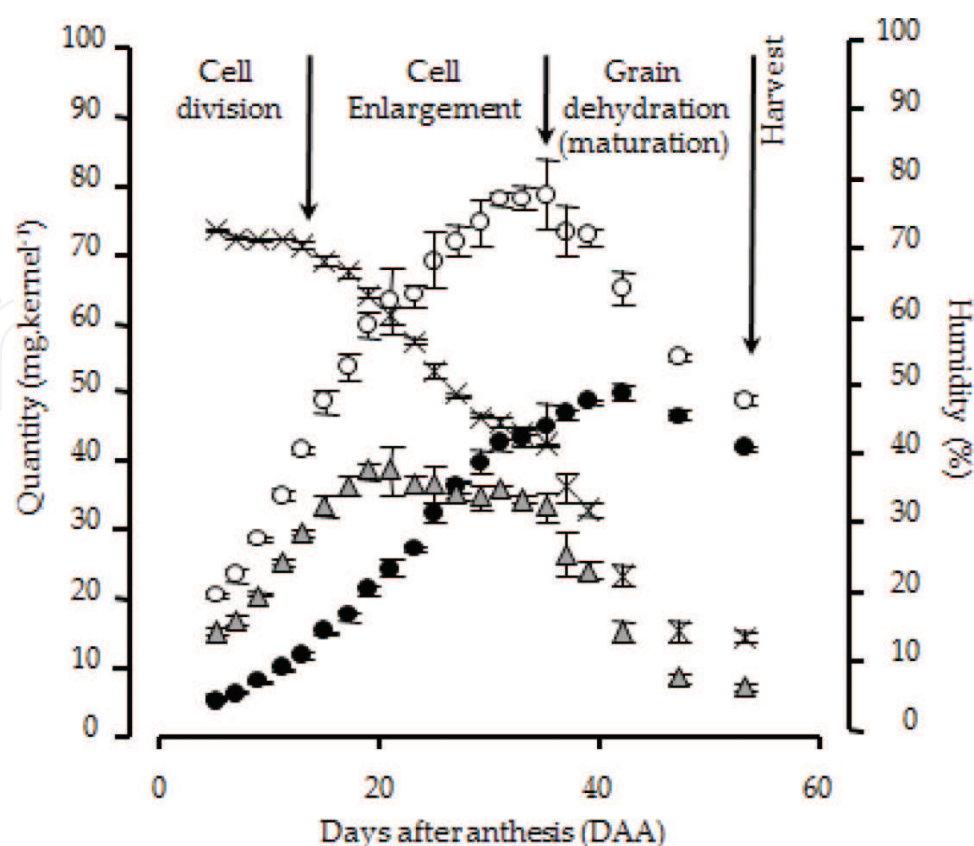
#### 3.1. Endosperm development

The development of the wheat endosperm which has been well described at the microscopic level, as reviewed by Bechtel et al. [66], can be quite easily characterized by the study of the temporal variations of several quantitative components of it such as the accumulation of the total dry matter, the water content of the grain and the accumulation of total protein and starch [67] (**Figure 7**).

The accumulation of total dry matter in the grain provides a good insight into the functioning of different accumulation metabolisms (i.e. nitrogen translocation and post-flowering photosynthesis) [68]. Thus, after an initial lag phase (up to 10–15 days after anthesis (DAA)), it is easy to observe a phase of linear accumulation of this dry matter; wheat grains reaching a maximum dry weight from 40 DAA.

During this linear phase, the observed phenomena depend on two main variables: the duration ( $D$ ) and the speed or flux of assimilates towards the grain ( $V$ ), so that the weight of a grain ( $P$ ) is given by the relation  $P = V \times D$  [69].  $D$  can be expressed in days or in the sum of average daily temperatures (i.e. degree-days ( $DD$ )). The filling speed is the limiting factor in the development of the weight of a grain. This speed is mainly by the number of grains per  $m^2$ . Finally, under natural conditions, the duration  $D$  cannot compensate for the weight loss produced by any reduction in the rate of accumulation. The amount of water per grain that gradually increases to about 20 DAA remains relatively constant up to  $\approx 35$  DAA (i.e. “water plateau” phase) before decreasing at harvest time.

The higher the rate of water accumulation in the grain, the greater the height of the “water plateau” and the higher the weight of the grain at maturity [70]. Based on changes in the amount of water and total dry matter per grain after anthesis, three particular phases of grain development can be estimated: the cell division phase, the cell enlargement phase (i.e. grain



**Figure 7.** Grain filling period for a common wheat cultivar (*Soissons*). Evolution of (●) dry matter per kernel, (○) fresh matter per kernel, (△) water quantity per kernel and (×) grain humidity. The vertical lines represent the standard deviation ( $n = 3$ ) (from Carceller and Aussenac [67]).

filling phase) and the grain desiccation phase (the beginning of this phase corresponding to the acquisition of physiological maturity) [71] during which protein bodies disappear to form the protein matrix [72, 73].

Since 1970s, a great deal of work has been done to evaluate the effects of the environment on grain development. Thus, the effects of several environmental variables (i.e. light, temperature, water availability and nutrient availability), taken individually or in combination, have been studied [74–85]. In general, temperature and water availability strongly affect the filling rate ( $V$ ) and the duration of grain filling ( $D$ ), although some differences in behaviour may exist between wheat genotypes. Consequently, differences in thermal regimes and/or water regimes cause profound changes in the accumulation of the total dry matter ( $P$ ) by affecting indifferently and without compensating the speed and duration of filling [86].

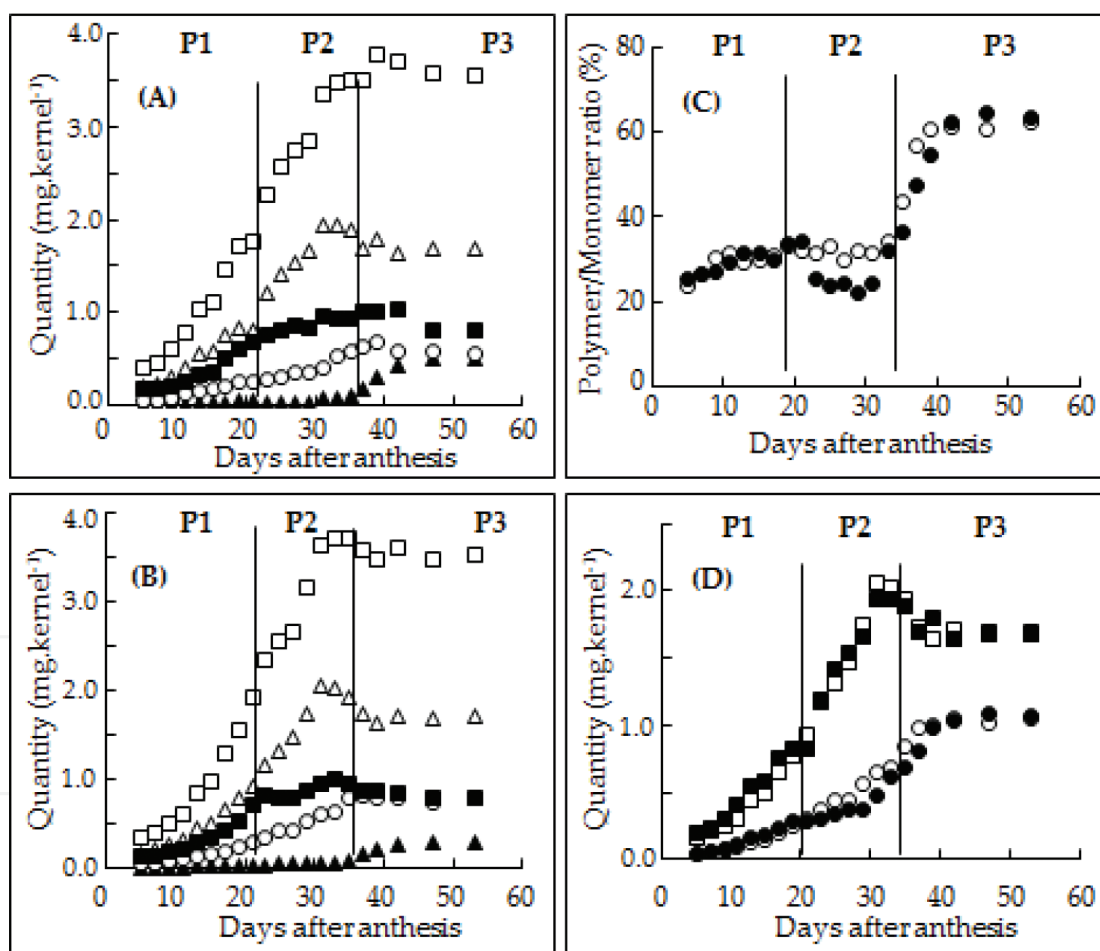
### 3.2. Accumulation of Storage Proteins

The accumulation of different protein fractions (albumins-globulins, gliadins and glutenins) is progressive from flowering until the acquisition of the physiological maturity of the grains ( $\approx 35$ – $40$  DAA). However, even if the time of initiation of the biosynthesis of the different proteins of the grain is not significantly different (5–7 DAA) [87], their rate of accumulation

varies considerably, suggesting a phenomenon of differential regulation of this biosynthesis (Figure 8A and B).

Thus, a certain accumulation asynchrony in the protein fractions of the grain can be highlighted. The albumins-globulins accumulate most rapidly in the grain, followed by the monomeric prolamins and finally the polymeric prolamins. As many researchers have shown [88–93], the accumulation of albumins-globulins is maintained only during the cell division phase, contrary to that of prolamins. This confirms the functional and/or structural role of these specific proteins.

While the ratio between polymeric proteins and monomeric proteins is stable during the first stages of grain development (i.e. cell division and cell enlargement), this ratio increases significantly during the grain desiccation phase (i.e. after 35 DAA) (Figure 8C). A number of results in the literature are quite contradictory [90, 94, 95]. In our opinion, and in accordance with the



**Figure 8.** Evolution of the quantity of the different protein fractions (mg.kernel<sup>-1</sup>) for two common French wheat cultivars (A) Soissons and (B) Thésée, as a function of the days after anthesis. (▲) SDS-insoluble polymers; (○) SDS-soluble polymers; (■) albumins and globulins; (△) monomers and (□) total proteins. Evolution of (C) the polymer/monomer ratio (%) and (D) the quantity of monomers and total polymers (mg.kernel<sup>-1</sup>) as a function of days after anthesis. (○,●) total polymers of Thésée and Soissons, respectively; (□,■) Monomers of Thésée and Soissons, respectively. Stages: (P1) cell division; (P2) cell enlargement and (P3) grain desiccation and maturation (from Carceller and Aussenac [67]).



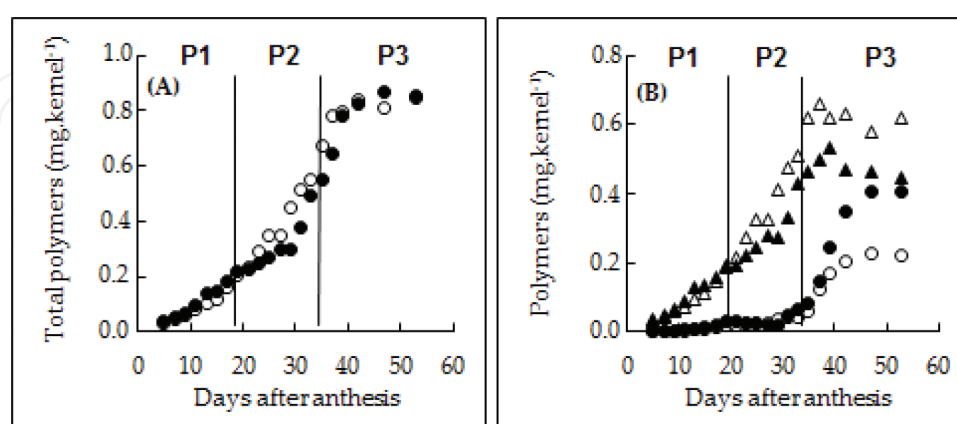
remarks of Stone and Nicolas [92], most of these differences can be explained by the fact that the methods of extraction and analysis of the polymeric proteins retained are extremely varied from one research group to another; it is therefore certain that all the researchers did not take into account the same protein entities in the calculation of the polymers/monomers ratio.

The accumulation of SDS-soluble polymers that starts very early in the grain (from 7 DAA), is very slow and continues up to the beginning of the drying phase of the grain. The accumulation of SDS-insoluble polymers (i.e. UPP) is, in turn, really visible only when the grain begins to lose its water balance (i.e. end of the “water plateau”) [67, 92, 96] (**Figure 9B**).

These elements must be compared with the observations of researchers such as Woodman and Engledow who, as early as the 1920s, noted the increase in the ability of proteins to form a coherent mass, gluten, in relation to the beginning of the grain desiccation [97]. The accumulation of the protein polymers in the broad sense coincides perfectly with the accumulation of the different glutenin subunits (LMW-GS and HMW-GS) in the grain [91, 98]; the HMW-GS/LMW-GS ratio being an important parameter for differentiating wheat genotypes from each other. For example, in the framework of our own research [67, 99], we have been able to demonstrate that at harvest time, the association state of polymeric proteins (i.e. SDS-insoluble polymers/total polymers ratio) is strongly correlated with the HMW-GS/LMW-GS ratio. Thus, at maturity, with the same total polymer amount (**Figure 9A**), the wheat genotype Soissons, which is characterized by a HMW-GS/LMW-GS ratio twice that of the wheat genotype Thésée, has a SDS-insoluble polymer/total polymer ratio twice as large that of Thésée (**Figure 9B**).

### 3.3. Unextractable polymeric protein (UPP) accumulation

The formation and accumulation of polymeric protein fractions characterized by high levels of aggregation (indifferently qualified in the literature of SDS-insoluble polymeric proteins, unextractable polymeric proteins (UPP) and glutenin macro polymers (GMP)) have been the focus



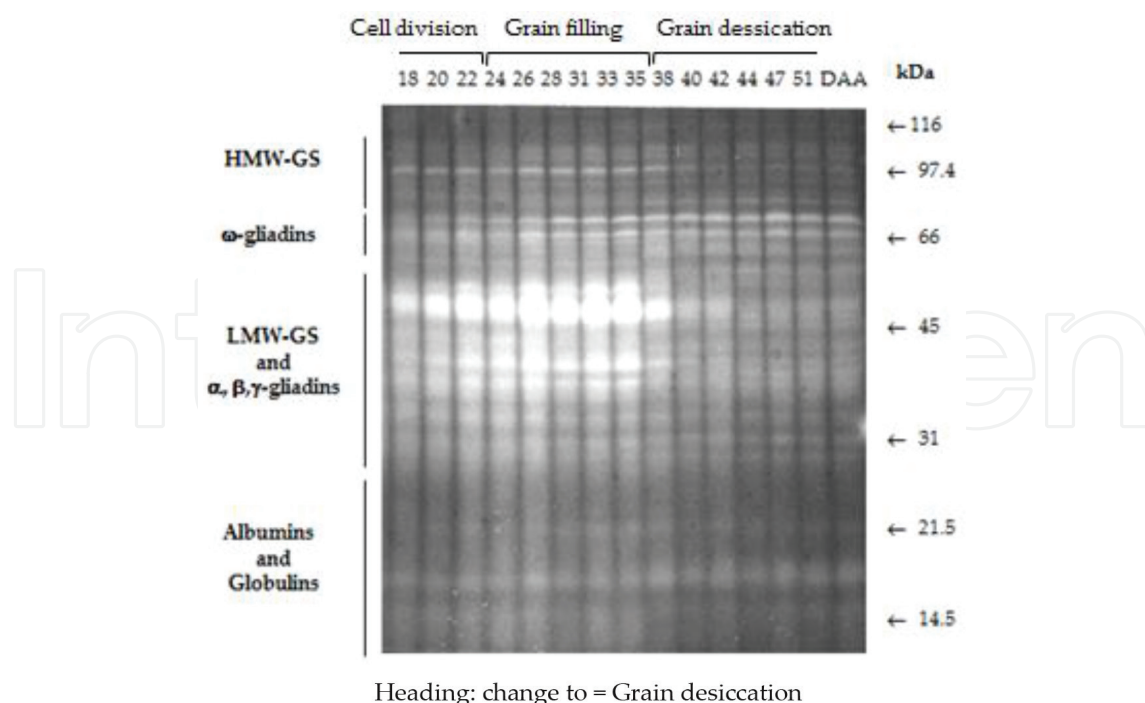
**Figure 9.** Accumulation of total polymers, SDS-soluble and SDS-insoluble polymers as a function of the days after anthesis. (A) Total polymers (mg.kernel<sup>-1</sup>) for (●) Soissons and (○) Thésée. (B) Open symbols are for Thésée and closed symbols are for Soissons. (△) SDS-soluble polymers and (○) SDS-insoluble polymers. Stages: (P1) cell division; (P2) cell enlargement and (P3) grain desiccation and maturation (from Carceller and Aussenac [67]).

of attention during the last 15 years because these fractions became widely recognized as the key protein fraction that can explain differences in dough strength and breadmaking quality.

According to the various physiological observations carried out since the early 2000s [67, 93, 100–102], it appears that the UPP accumulation phase coincides very strongly with the grain desiccation phase (**Figure 9B**), whatever the culture conditions applied (i.e. light, temperature, water availability and nutrient availability). Thus, 95–100% (w/w) of the UPPs present in the grain at harvesting accumulates during the grain desiccation phase. Finally, several experiments of artificial dehydration of wheat grains have confirmed the strong relationship between grain water loss and UPP accumulation [93, 102].

Although today the mechanisms responsible for the formation of UPPs are still the subject of discussions and/or hypotheses, many observations seem to confirm that the strengthening of the aggregation character in these polymeric proteins during grain desiccation results from the reinforcement of intermolecular interactions (mainly covalent interactions) between the different glutenin subunits (HMW-GS and LMW-GS) [103, 104]. This phenomenon has led to a very significant increase in the different molecular dimensions (Mw and Rg) of the glutenin polymers [103].

Studying the function of free glutenin sulfhydryl (SH) and disulphide (SS) groups in glutenins of developing wheat for UPP formation, we showed that the major wheat glutenin subunits residing in the protein bodies undergo redox change during the development and the maturation of the grain [103] (**Figure 10**). Indeed, during the cell division and grain filling, glutenin



**Figure 10.** Change in sulfhydryl status of wheat proteins during grain development and maturation. MBBBr-derivatized (fluorescence photography) storage proteins of a common French wheat cultivar (Soissons). Days after anthesis (DAA) (from Rhazi et al. [103]).

subunits and particularly LMW-GS have a large amount of free SH groups and become oxidized during grain desiccation which coincided with the accumulation of UPP. Moreover, monobromobimane (mBBBr) derivatized of free glutenin SH groups before the artificial grain desiccation totally inhibits the UPP deposition [104].

In our hypothesis which is very close to the model proposed by Hamer and van Vliet [105] for the gluten structure termed “hyper aggregation” model, the grain desiccation promotes the aggregation of polymers already present (i.e. SDS-soluble polymers or level I aggregates in the “hyper aggregation” model) by facilitating specifically the formation of interchain hydrogen bonding between the repeat regions of glutenin subunits [106–108], which can bring glutenin free accessible SH groups into close proximity to form additional intermolecular disulphide bridges.

4. Impacts of environmental factors on MWD of prolamins

The multiple agronomic studies which were done during the last 25 years indicate that environmental conditions affect the amount, composition and polymerization of the gluten proteins [109–119]. Furthermore, the impact of environmental components on the molecular weight distribution of the prolamins is significantly greater than that of genetic components (i.e.  $\sigma^2_E/\sigma^2_R > \sigma^2_G/\sigma^2_R$ ) (Table 2) [120–122]. This is why, in a context of profound environmental changes [123], it is very important to better understand the mechanisms responsible for these effects in order to better anticipate them.

The availability of nutrients (nitrogen and/or sulphur availability) and the temperature (thermal regime) are the two main environmental factors responsible for these protein changes.

Parameters	Maximum value	Minimum value	Mean value	$\sigma^2_G/\sigma^2_R$	$\sigma^2_E/\sigma^2_R$
Total proteins <sup>1</sup>	8.700	15.100	11.187	11.411***	271.577***
Total polymers <sup>1</sup>	2.785	5.755	4.016	11.104***	187.462***
Polymer/monomer	0.321	0.700	0.561	14.845***	72.611***
Polymer $M_n^2$	$0.730 \times 10^6$	$9.609 \times 10^6$	$0.972 \times 10^6$	1.068 <sup>NS</sup>	4.383***
Polymer $M_w^2$	$1.142 \times 10^6$	$22.970 \times 10^6$	$7.640 \times 10^6$	3.370***	38.974***

<sup>1</sup>Quantity in mg/100 g DM.  
<sup>2</sup> $M_n$  = Molecular weight number-average and  $M_w$  = Molecular weight-average (g.mol<sup>-1</sup>).  
\*\*\*F-test significance at 0.1% level of probability;  $\sigma^2_G/\sigma^2_R$  = Genetic variance/Residual variance ratio and  $\sigma^2_E/\sigma^2_R$  = Environmental variance/Residual variance ratio.  
NS: Not significant.

**Table 2.** Genetic (G) and environmental (E) influence on molecular weight distribution of storage proteins determined by analysis of variance (F-test) for 130 common French wheat genotypes cultivated in three different locations for 2005 and 2006 (from Aussenac et al. unpublished data).

High nitrogen availability translates into high protein contents in the grain and flour but also by changes in protein composition. With increasing protein content, gliadins tend to increase at a greater rate than other proteins. This can lead to MWD alterations which results from decreases in the polymeric-to-monomeric protein ratio and/or increases in the HMW-GS to LMW-GS ratio [113, 124, 125].

When sulphur fertilization is limited, the molecular distribution of glutenins is strongly affected insofar as this limitation results in a significant modification of the HMW-GS/LMW-GS ratio [109, 126]. The increase in the HMW-GS/LMW-GS ratio which is linked to the fact that the high molecular weight glutenin subunits are much less affected by a sulphur limitation because they are poorer in corresponding amino acids, therefore results in an increase in the average molecular weight of the polymers. Finally, sulphur deficiency is accentuated by higher nitrogen levels [127].

Temperature (i.e. daily mean temperature, temperature regime and temperature application stage) can induce very large changes in the association state of polymers during grain filling [110, 128–130]. Thus, in the great majority of the work carried out in recent years, various researchers have shown that the increase in temperature and/or the sudden change in the thermal regime during grain filling could lead to a significant decrease in the association status of prolamins resulting in a decrease of MWD (or solubility) of glutenins [131–133].

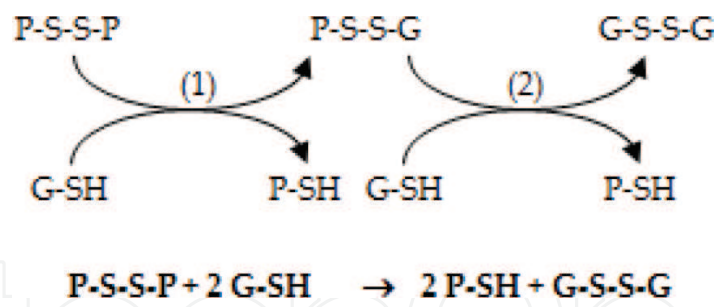
In the majority of the work to which we have just referred to above, the effects observed are most often attributed to modifications in the synthesis activities of the different storage proteins (i.e. gliadins vs. glutenins and/or HMW-GS vs. LMW-GS) resulting from modulation of the expression of storage protein genes [85]. Today, it seems that other phenomena could also be reasonably involved. These phenomena could be based, in particular, on important variations in the cellular redox status in response to environmental stimuli (i.e. environmental stress).

It has long been established that desiccation of plant tissues causes the appearance of free radicals. Although this phenomenon is a very general mechanism, a large number of observations have been made from seeds of various species [134–137]. In the majority of these studies, the presence of free radicals has been correlated with viability losses [138]. Among these implemented detoxification mechanisms, the ascorbate/glutathione cycle (i.e. trapping of  $H_2O_2$  generated) is one of the most efficient. This essential cycle in chlorophyll tissues [139, 140] has also been studied in seeds [141, 142].

At a cellular level, thiols are the first compounds affected by oxidative stress in general because of the high sensitivity to the oxidation of sulfhydryl (SH) groups. The predominant non-protein thiol in most plant species is glutathione (GSH). This tripeptide ensures the maintenance of the redox status at a cellular level but also the storage and transport of the reduced sulphur necessary for the synthesis of proteins [143–145]. The first compound resulting from the oxidation of glutathione is its dimer (GSSG) which is produced *in vivo* largely thanks to SH/SS exchanges with proteins (noted P) [146]. The reactions below illustrate these exchanges.

The GSSG dimer is normally reduced in GSH by glutathione reductase (GR) activity. Thus, under normal conditions, glutathione is very much present at a cellular level in its reduced

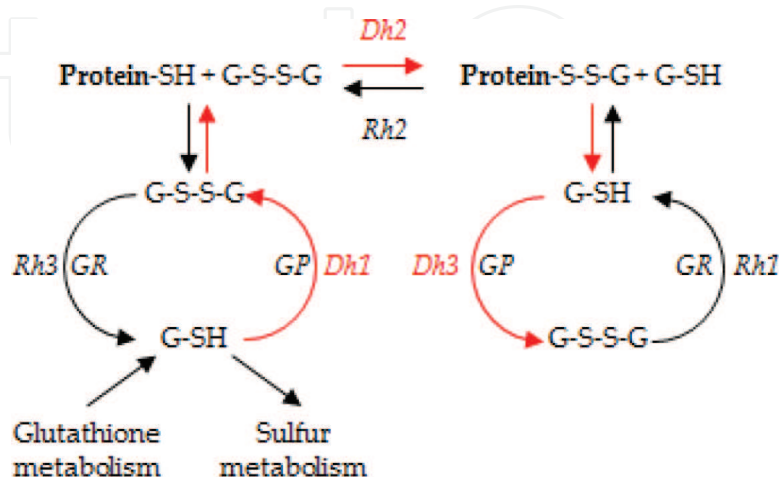




form (i.e. high GSH/GSSH ratio) which has the effect both for maintaining the SH status of proteins (to maintain enzymatic activities [147]) and continue to trap  $\text{H}_2\text{O}_2$ .

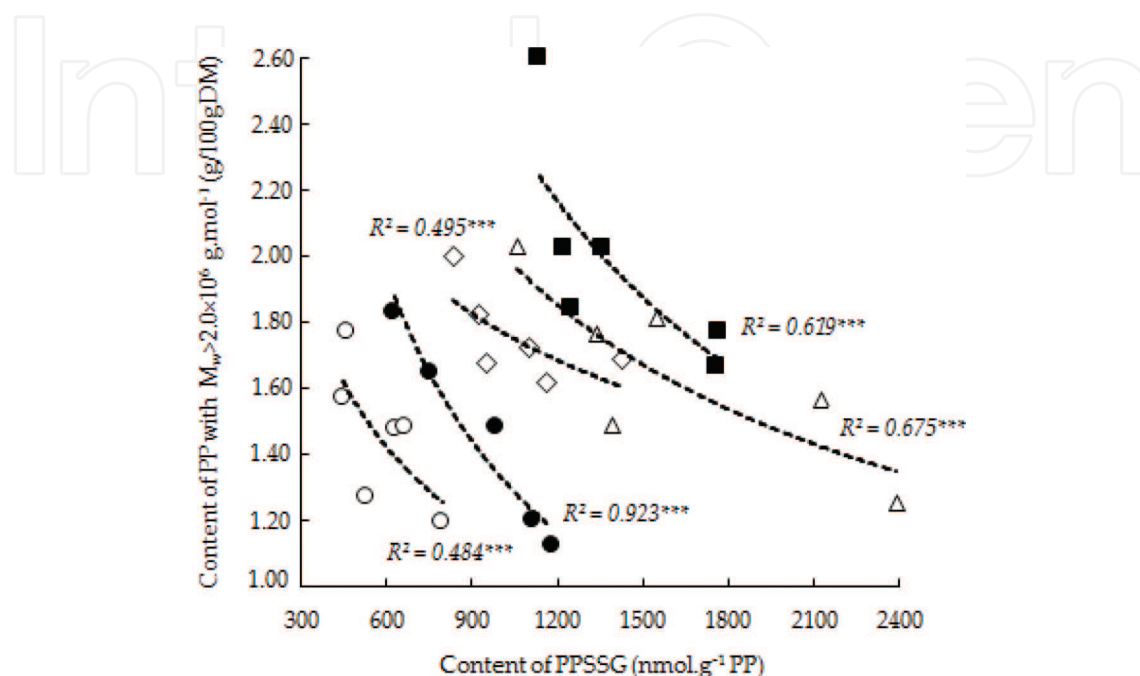
Under the influence of oxidative stress, the redox status of glutathione will be modified; GSSG dimer will accumulate due to either an increase in GSH oxidation and/or a decrease in GSSG reduction activity (i.e. decrease of GSH/GSSH ratio). Such changes in the SH/SS status have already been widely observed in response to oxidative stress, especially during seed desiccation [148]. Glutathione which is able to bind to protein thiols is considered a “protective” element of these protein compounds since it prevents the formation of intramolecular disulphide (S-S) bridges during the desiccation phenomena [149]. In this way, GSH contributes both to limit the protein denaturation phenomena and to modulate enzymatic activity [150]. In contrast to desiccation, the imbibition phenomenon preceding germination causes the reduction of the disulphide bonds (SS) of a large number of compounds such as, GSSG [151, 152], protein-SSG conjugates [153],  $\alpha$ -amylases [154] or the storage proteins [155, 156]. A synthesis of the presumed role of glutathione can be postulated, referring in particular to the hypothesis formulated by Kranner and Grill [150] (Figure 11).

Glutathione may occur endogenously in wheat flour in the free reduced glutathione (GSH) and free oxidized glutathione disulphide (GSSG) forms as well as in the form of protein-glutathione mixed disulphides (PSSG) [146–159]. Moreover, approximately 85% of PSSG in mature wheat grains are represented by polymeric proteins (PP) conjugated to glutathione

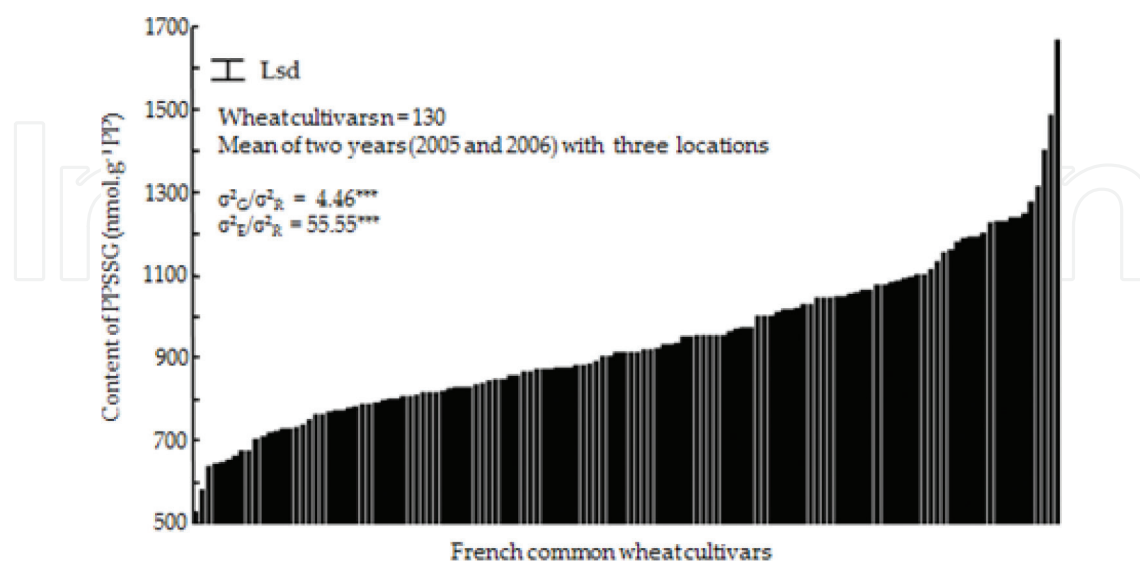


**Figure 11.** SH/SS interchange during dehydration/rehydration phenomenon. (*Dh*) dehydration step, (*Rh*) rehydration step, (*GP*) glutathione peroxidase, (*GR*) glutathione reductase (from Kranner and Grill [150]).

(PPSSG) [159]. Even if glutathione is able to bind to the storage proteins during grain filling, the formation of PSSG is not however correlated with the accumulation of the storage proteins in the grain but coincide rather with the grain desiccation during which the major wheat storage proteins residing in protein bodies undergo redox change (i.e. become oxidized) and UPP are accumulated [103, 159, 160].



**Figure 12.** The relationship between the content of high aggregated polymeric proteins (PP with  $M_w > 2.0 \times 10^6$  g.mol<sup>-1</sup>) and the content of polymeric proteins conjugated to glutathione (PPSSG) for five different common French wheat cultivars (harvest 2005 and 2006 in three locations) (from Aussenac et al. Unpublished data).



**Figure 13.** Variation of the content of polymeric proteins conjugated to glutathione (PPSSG) for a significant set of common French wheat cultivars (harvest 2005 and 2006 in three locations).  $\sigma^2_G / \sigma^2_R$  = genetic variance/Residual variance ratio and  $\sigma^2_E / \sigma^2_R$  = environmental variance/residual variance ratio (from Aussenac et al. Unpublished data).

Low molecular weight endogenous thiols such as glutathione, which mainly act as “protein protectors” [149] through the formation of PSSG during tissue desiccation, are responsible in wheat grains during its desiccation to a significant reduction of the MWD of the polymeric proteins by the formation of PPSSG (**Figure 12**). This action is all the more important because it is very targeted because GSH was bound almost exclusively to those cysteine residues that have been proposed to form intermolecular disulphide bonds (in particular, cysteines Cb\* and Cx, which are responsible for the aggregative nature of LMW-GS) as Köhler et al. [161] has been able to demonstrate it by using <sup>35</sup>Slabelled GSH.

Consequently, it is now clear that glutathione conjugation with polymeric proteins during the grain development resulting in drastic changes of the cellular redox status (largely due to environmental factors - **Figure 13**) plays a crucial role in controlling the MWD of the polymeric proteins which has been shown to be important in determining baking performance.

## 5. Conclusions

Since the 1990s, there has been a broad consensus within the scientific community that the value of using of a wheat flour depends mainly on the quality of the assembly of its prolamins (glutenins in particular) which are themselves largely under the control of protein polymorphism (the nature and relative abundance of LMW-GS and HMW-GS) and the conditions of development and maturation of the grains from which it is made. Although much progress has been made in the field of characterization of polymeric structures, in particular through the implementation of new analytical approaches (A-4F/MALLS), the fact remains that significant work needs to be done to better understand the structure of its protein assemblies of technological interest (UPP or GMP).

This chapter demonstrates that to achieve these objectives, it is essential to better understand the mechanisms that govern the formation of these polymers and/or protein aggregates in wheat grains during the final stages of their development which are subject to changing environmental conditions (i.e. rising temperatures). In this context, the important role of cellular redox status is addressed by highlighting the significant effects of particular free thiols such as glutathione on the state of association of glutenins. These compounds, of which one of the main functions is to limit the deleterious effects of oxidative stress on protein structures by combining with them, will at the same time reduce the inter-prolamin interactions in the grain thus limiting their technological functionalities. The current improved understanding of these cellular mechanisms will undoubtedly open up new avenues for exploring redox strategies for wheat improvement required for a sustainable quality.

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